J. Mountain Res. P-ISSN: 0974-3030, E-ISSN: 2582-5011 DOI:https://doi.org/10.51220/jmr.v18i2.19
Vol. 18(2), (2023), 173-180

Cyanobacterial Extracellular Polymeric Substances and their role in Biodeterioration of Temples and Monuments

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Received: 30.07.2023; Revised: 14.12.2023; Accepted: 16.12.2023

Abstract: Cyanobacteria are the ancient gram-negative prokaryotic organisms having a wide range of forms ranging from unicellular to filamentous forms. They are oxygenic photosynthetic microorganisms having cosmopolitan distribution in almost all aquatic, terrestrial habitats and even in extreme ecosystems (e.g., hot and cold deserts, hydrothermal vents, hypersaline environments and rocks) due to their tremendous adaptability to varying environmental conditions. Extracellular Polymeric Substances (EPS) are the extracellular mucilaginous substances produced by members of cyanobacteria. Production of EPS is widely known in cyanobacteria and protect the cyanobacterial cells from various stresses such as desiccation and UV-radiation. This review discusses about cyanobacterial EPS and its role in biodeterioration of monuments and temples.

Keywords: Extracellular Polymeric Substances • biodeterioration.

Introduction

Cyanobacteria (Blue-green algae) are an evolutionary ancient group of gram-negative, prokaryotic, oxygenic photosynthetic microbes inhabiting a wide range of habitats including extreme environments such as cold and hot deserts, dry rocks, stone-built structures, hypersaline environments etc. They are equipped with a number of adaptive features and protection mechanisms. Mostly cyanobacterial cells and filaments are surrounded by a mucilaginous matrix which is known as Extracellular Polymeric Substances (EPS). Chemically, majority of EPS is polysaccharidic in nature, composed of various homopolysaccharides and heteropolysaccharides.

Extracellular Polymeric Substances (EPS) are the natural polymer of high molecular weight compounds, produced by various microorganisms, which are synonymously called as Exopolysaccharides. Cyanobacterial EPS can be categorised into two types- one associated with cell surface as Capsular Polysaccharides (CPS) and other polysaccharides released into the surrounding environment as Released Polysaccharides (RPS). The capsular polysaccharides (CPS) may be referred to as capsule, sheath or slime depending on their thickness, consistency and appearance (De Phillipis and Vincenzini, 1998, 2003; Potts, 2004; Kumar *et al.*, 2018). Sheath is a thin and dense layer loosely surrounding the cells or cell groups and can be seen microscopically without staining. The capsule generally consists of thick and slimy layer tightly associated with the cell surface with sharp outlines reflecting shape of the cell. The slime refers to the dispersed mucilaginous material around the organisms without reflecting shape. The EPS of cyanobacteria have always remained the substance of attraction due to industrial significance as/in food additives, food thickeners, cosmetics, soil stabilizers and bioremediation of pollutants. EPS have several roles ranging from structural roles (composition of biofilm matrix) to the functional roles (cell-cell interaction, protective roles in desiccating environment, defence roles, site receptor for phage and bacteriocin etc). Production of EPS and other peculiar features make cyanobacteria the key organisms in process of formation of biofilms on lithic substrata (Stal, 2000).

EPS maintain the structural and functional integrity of the microbial biofilms (Flemming *et al*., 2000). Ten monosaccharides have been reported in cyanobacterial EPS, including hexoses (glucose, galactose, mannose), pentoses (ribose, xylose, arabinose), deoxyhexoses (fucose, rhamnose), and acid hexoses (glucuronic acid and galactouronic acid) (De Philippis and Vincenzini, 1998). EPS either remain attached to the cell's outer surface or secreted outside the cell. EPS constitute 50 to 90% of a biofilm's total organic matter (Flemming *et al*., 2000; Donlan, 2002; Donlan and Costerton, 2002). Several factors are known to influence the composition of EPS such as, species, substratum type, nutrient availability, temperature, pH, light intensity (Cheah and Chan, 2021).

This review focusses on the EPS produced by the rock-dwelling cyanobacteria growing on the natural rocks and man-made buildings and their effects in the deterioration of the temples and monuments.

Occurrence of cyanobacteria on Temples and Monuments

Cyanobacteria in association with other microbes (e.g., green algae, bacteria, fungi, lichen) have been reported on temples, monuments and ancient buildings in India as well as in other countries by several researchers (Büdel, 1999; Crispim and Gaylarde, 2005; Crispim *et al*., 2003; Crispim *et al*., 2006; Pandey, 2011; Adhikary, 2000; Mandal and Rath, 2012; Bhavani *et al*., 2013; Samad and Adhikary, 2008; Tripathi *et al*., 1999; Pradhan *et al*., 2018). A good number of cyanobacterial genera and species have been reported as epilithic organisms growing and surviving on exposed surfaces of stone-built monuments and temples. The rock-inhabiting organisms occurring on or within the rocks are called as lithobionts, lithobiontic or lithophytic organisms. They are classified into various groups based on the location where they live. Organisms that grow attached to the external surfaces of rocks are known as 'epiliths' or 'epilithobionts', and those that grow inside rocks are known as 'endoliths.' The presence of hygroscopic extracellular polymeric substance (EPS) has been shown to be involved in adhesion of cyanobacterial cells to the lithic surfaces and biofilm formation (Cecchi *et al*., 2000; Warscheid and Braams, 2000; Decho *et al*., 1990). EPS exert pressure during volume changes due to hydration and dehydration cycles and act as possible factor in rock weathering (Jaag, 1945; Friedmann, 1971; Golubic, 1973; Anagnostidis *et al*., 1983). Also, the role of epilithic cyanobacteria in the dissolution of calcium carbonate in nature should not be ignored (Viles *et al*., 1987). Bioreceptivity, the ability of a particular substratum to be colonized by organisms, of rock and building surfaces depends on surface condition, roughness, mineral composition, porosity, and permeability (Guilitte and Dressen, 1995; Gaylarde *et al*., 2003).

EPS production as a metabolic strategy against environmental stresses

The rock-inhabiting cyanobacteria (lithic cyanobacteria or lithobiontic cyanobacteria) face a range of environmental stresses on exposed surfaces of rocks, such as high intensity of solar radiation, high temperature, desiccation, oxidative stress, nutrient unavailability etc. Against desiccation stress, EPS plays important role in providing suitable microenvironment by acting as a water/moisture reservoir. Cyanobacteria-dominated biofilms, which are also called crusts or patina, cause unpleasant discoloration of the surfaces of monuments and temples due to the photosynthetic pigments and other metabolic products.

Many microorganisms produce large amounts of exopolysaccharides (De Philippis and Vincenzini, 1998). Excess carbon availability and deficiencies in nitrogen, potassium, and phosphate lead to high production of EPS (Sutherland, 2001). The hygroscopic nature of EPS contributes to the desiccation tolerance of microorganisms. EPS forms a hydrated boundary between a microbial cell and its surrounding environment (De Philippis and Vincenzini, 1998).

EPS plays both structural and functional roles in cyanobacteria such as maintenance of colony structure, biofilm formation, surface adhesion and colonization, and protection of cells from various types of stresses which include dehydration (Potts, 1994; Potts, 1999; Tamaru *et al*., 2005), freezing (Knowles and Castenholz, 2008; Tamaru *et al*., 2005) and UV radiation (Adhikary and Sahu, 1998; Garcia-Pichel and Castenholz, 1991). In aero-terrestrial lithic habitats, lithobiontic cyanobacteria are subjected to repeated cycles of drying and rewetting. Due to its hygroscopic nature, EPS can absorb and retain water, creating a moist microenvironment around cyanobacterial cells necessary for drought tolerance (Rossi and De Phillipis, 2015). Many studies have reported the presence of the UV-absorbing pigment scytonemin within the envelope of cyanobacteria (Keshari and Adhikary, 2013; Adhikary and Sahu, 1998; Garcia-Pichel and Castenholz, 1991). Cyanobacterial EPS are anionic (negatively charged) due to the presence of sulphate groups, glucuronic acid, and galacturonic acid. Due to its anionic nature, EPS has an affinity for cations (positively charged ions) such as Ca^{+2}, Mg^{+2}, Fe^{+2} and various metal ions (Rossi *et al*., 2012; De Philippis and Vincenzini, 2003).

Effects of cyanobacterial EPS on lithic substrata of Monuments and Temples

As shown in the Fig. 1, EPS play structural and protective role in cyanobacteria and cause deteriorating effects on lithic substrata. Microbial-induced biological weathering or biodeterioration of stone-built structures is a complex process in which different microorganisms interact with stone and building materials and the environment. Various studies conducted around the world have revealed the role of lithobiontic cyanobacteria in the biodeterioration of monuments and historic buildings (Crispim *et al*., 2003; Crispim *et al*., 2004; Crispim and Gaylarde, 2005; Gaylarde and Gaylarde, 1999; Gaylarde *et al*., 2007; Gaylarde *et al*., 2012; Gaylarde and Morton, 1999; Gaylarde, 2020; Ortega-Morales *et al*., 2000; Macedo *et al*., 2009; Kovacik, 2000; Adhikary and Kovacik, 2010; Samad and Adhikary, 2008). John(1988) made literature review focusing on the algae and cyanobacteria that grow on monuments and buildings worldwide and the factors promoting their establishment and growth. These include various stone monuments, buildings, architectural structures and artifacts such as cathedrals, chapels, churches, monasteries, mosques, temples, palaces, pyramids, historic buildings, statues and tombs.

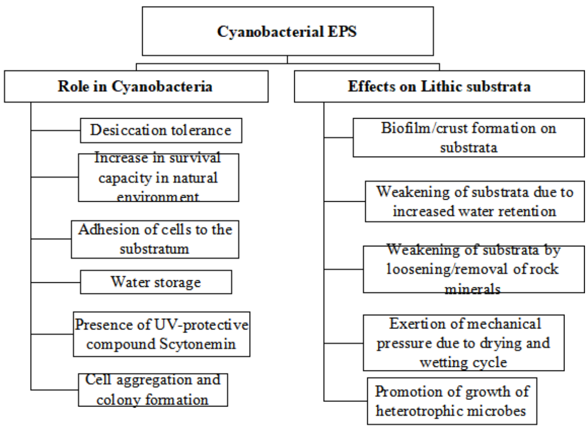

Figure 1. Role of EPS in survival of cyanobacteria on rock (lithic) substrata and its effects on lithic substrata

The EPS of cyanobacteria- dominated biofilms formed on the exposed surfaces of stone monuments and temples readily absorb the available water. The absorbed water is not lost by evaporation as rapidly as it would from rock surfaces without biofilms. Retained water may accelerate aqueous chemical reactions, causing weathering and degradation of stone (Gorbushina, 2007). Water and moisture weaken rocks/stones and affect various mechanical properties (Hawkins and McConnell,1992; Vásárhelyi and Ván, 2006; Erguler and Ulusay, 2009). Water causes hydrolysis of silicate minerals, dissolution of carbonates and the formation of gypsum crusts (Hall *et al*., 2011). Additionally, the biofilms formed as a result of EPS have lower thermal conductivity than stone, which can lead to uneven heat transfer within the structure (Warscheid and Braams, 2000). The discoloured/stained areas produced on the surface absorb more solar radiation, resulting in an increase in surface temperature (Garty, 1990). A temperature or thermal gradient induces physical stress through expansion and contraction (Warscheid, 2000). The presence of cyanobacterial biofilms on exposed stone surfaces may facilitate the accumulation of airborne contaminants (Steiger *et al*., 1993; Witteburg, 1994). Some of the accumulated contaminants may serve as a nutrient source for microbial colonization (Nuhoglu *et al*., 2006). EPS acts as a source of organic carbon for the growth of heterotrophic microbes viz. bacteria and fungi which also contribute to the biodeterioration of lithic monuments and temples.

Production of EPS, siderophores, or other chelating agents and acid or alkaline secretion by microorganisms are implicated in bioweathering of natural stones and building stones through chelating and solubilizing effects on rock minerals (Gaylarde and Gaylarde,1999; Ortega- Morales *et al*.,2000; Wessels and Büdel, 1995). Due to its anionic nature, EPS is able to bind/chelate cationic metals of minerals in lithic substrates (Welch and Vandevivere, 1994; Rossi *et al*., 2012).

Conclusion

The extracellular polymeric substances (EPS) produced by cyanobacteria are very important for their survival on rock substrata, but have deteriorating effects on natural stones or stone-built monuments, temples and other structures. Cyanobacteria are considered as an important group of biodeteriogens of monuments, temples and buildings. Being photoautotroph and diazotrophs (in many cases), they have simple growth requirements which make them pioneer community in many habitats where they grow and survive. The Extracellular Polymeric Substances (EPS) produced by cyanobacteria cause the weakening of the rock substrata due to water retention, metal chelation and pressure exertion due to volume changes. EPS ensure the longer survival of cyanobacteria under the adverse conditions on exposed rock surfaces. EPS production strategy of cyanobacteria to protect from desiccation on rocks plays an important role in the biodeterioration of temples and monuments. Monuments are priceless assets that represent rich cultural and historical legacy of a society and a country. They represent human sensitivity, creativity, and belief, and are important from cultural, historical, archaeological and religious point of view. Constructed from diverse materials, including stones (such as sandstone, limestone, marble and granite), bricks, concrete, and mortars, they exhibit an array of sculptures, styles and embellishments. Temples and monuments are the heritage structures having religious, cultural, historical and economic significance. Globally, their deterioration/ biodeterioration is a matter of serious concern for historians, scientists, environmentalists, conservators and policy makers. Research should be directed towards the development of effective methods for the control of biological weathering or biodeterioration caused by cyanobacteria and other organisms or by their metabolic products.

Acknowledgment
The authors are thankful to the Principal, Pt. L.M.S. Govt. Post-Graduate College, Rishikesh, for providing necessary infrastructure and facilities. Rashmi Kala is grateful to the Council of Scientific & Industrial Research, New Delhi, India for awarding Research Fellowship for doctoral research.

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